CODING FORM FOR SRC INDEXING **REVISED 10/15/86**

Microfiche No.

OTS0534399

New Doc I.D.

86-920000194

Old Doc I.D.

Date Produced

1/01/81

Date Recieved

11/27/91

TSCA section

8D

Submitting Organization

MOBAY CORP

Contractor

INVERESK RES INTL LTD

Document Title

TESTING THE MUTAGENIC ACTIVITY OF HE 1003 IN THE MOUSE LYMPHOMA MUTATION ASSAY (FINAL REPORT) WITH COVER LETTER DATED 112291

Chemical Category

POLYMETHYLENE POLYPHENYLENE ISOCYANATE (9016-87-9)

CONTAINS NO CBI

Mobay

91 NOV 27 AM 9:38

A Bayer USA INC COMPANY

Mobay Corporation
Health, Environment, Safety
& Plant Management

Mobay Road
Pittsburgh, PA 15205-9741
Phone: 412 777-2000

November 22, 1991

Document Processing Center TS-790
Office of Toxic Substances Room L-100
Environmental Protection Agency
401 M Street SW
Washington, DC 20460

8920000194

86920000194

Attention: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Gentlemen:

Enclosed are copies of Health and Safety Stu[illegible] submitted on behalf of Mobay Corporation, Mobay Road, Pittsburgh, Pennsy[illegible]15205. We are filing these Health and Safety Studies to comply with the [illegible]lations codified at 40 CFR, Part 716. This submission contains no Confidential Business Information (CBI).

The information required at 40 CFR 716.30 is given below.

Chemical Name:	Polymethylene polyphenylene isocyanate
CAS No:	9016-87-9
Name of Study:	Testing the Mutagenic Activity of HE 1003 in the Mouse Lymphoma Mutation Assay
Submitting Official:	Francis J. Rattay
Title:	Manager, Regulatory Compliance
Address:	Mobay Road Pittsburgh, Pa 15205
Telephone No.:	(412) 777-7471
FAX No.:	(412) 777-7484

If you have any questions, please contact me.

Sincerely,

Francis J. Rattay
Manager, Regulatory Compliance
(412) 777-7471

Attachments
Certified Mail No.: P 276 377 332

R 2098

CONTAINS NO CBI 24407

TESTING THE MUTAGENIC ACTIVITY OF HE 1003 IN THE MOUSE LYMPHOMA MUTATION ASSAY

IRI Project No. 703837

I.R.I. Inveresk Research International

INVERESK RESEARCH INTERNATIONAL
Report No. 1883

CONFIDENTIAL

TESTING THE MUTAGENIC ACTIVITY OF HE 1003 IN THE MOUSE LYMPHOMA MUTATION ASSAY

IRI Project No. 703837

Authors:

D.B. McGregor
W.J. Harris
C.A. Ross

To:

Bayer AG
Institut für Toxikologie
Friedrich-Ebert-Strasse
217-319
Wuppertal 1
West Germany

Issued by:

Inveresk Research International
Musselburgh
EH21 7UB
Scotland

6658

January 1981

AUTHENTICATION

"I, the undersigned, hereby declare that this work was performed under my supervision, according to the procedures herein described and that this report represents a true and accurate record of the results obtained."

W.J. Harris, B.Sc., Ph.D.
Principal Investigator

Project No. 703837

Report No. 1883

QUALITY ASSURANCE AUTHENTICATION

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a pre-determined schedule.

The report has been audited by IRI Quality Assurance personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

IRI PROJECT NO. 703837 Report No. 1883

Signed Andrew Wooldeff Date 23rd September 1981.

Quality Assurance Manager

CONTENTS

PERSONNEL INVOLVED IN PROJECT 703837

Division Head:	D.B. McGregor, B.Sc., Ph.D.
Principal Investigator:	W.J. Harris, B.Sc., Ph.D.
Project Leader:	C.A. Ross, B.Sc., Ph.D.
Technical Assistance:	J. Liddle N.R. Hunter, H.N.D. C. Green, B.Sc. J. Harvey, B.Sc.
Quality Assurance:	A.W. Waddell, B.Sc., Ph.D. E.M. Baxendine, B.Sc.

SUMMARY

The compound HE 1003 was tested for potential mutagenicity in the mouse lymphoma L5178Y specific locus mutation test. The primary criterion used for a significant positive effect in this test was a doubling of the mutation frequency at the thymidine kinase locus over a solvent treated negative control. Mutation was scored as resistance to trifluorothymidine.

An initial toxicity test was carried out in the absence of a post-mitochondrial supernatant fraction from the livers of Aroclor 1254-treated adult male rats and the co-factors required for mixed function oxidase activity (S-9 mix) and with a dose range of 1060 µg/ml to 0.1 µg/ml test compound. HE 1003 killed all the cells at 1060 µg/ml; and a top dose of 100 µg/ml was chosen for the first mutation test.

Three mutation tests were carried out both in the absence and the presence of S-9 mix. In both the absence and presence of S-9 mix, HE 1003 showed no evidence of mutagenic activity in the mouse lymphoma mutation assay.

INTRODUCTION

The mutagenicity of compound HE 1003 was examined in the L5178Y mouse lymphoma mutation test. This test, which measures mutation frequencies at a specific locus in cultured mammalian cells, provides a convenient experimental system for testing potential environmental mutagens. Advantageous characteristics of the system include an ability to grow in suspension culture, a short generation time and a cloning efficiency near 100% in a simple soft agar cloning medium.

The mouse lymphoma cells used in the assay were those of strain L5178Y rendered heterozygous at the normally diploid thymidine kinase (TK) locus. Mutation from $TK^{+/-}$ to $TK^{-/-}$ was measured using a modification of the method of Clive et al., (1972;1977). The growth of mutant colonies in soft agar containing trifluorothymidine, which is non-toxic to cells lacking thymidine kinase, indicated that mutation had occurred at the TK locus. A validation of this system had recently been published (Clive et al., 1979). This validation consisted of dose response data with 43 chemicals which represent weak and potent mutagens and carcinogens which are weakly or non-mutagenic in the Ames bacterial mutation assay. Several carcinogens which are negative or difficult to detect in the standard Ames assay are mutagenic in the mammalian cell system.

The tests described in this report were conducted in the Inveresk Gate Laboratories of Inveresk Research International Limited. They were carried out between 19 August and 27 October 1980.

MATERIALS AND METHODS

Sterile procedures were used throughout preparation of materials and experimental methods.

Chemicals

The compound HE 1003 was received from Bayer AG on 15 August 1980. It was described as an oily brown liquid and kept at 4°C in a metal container.

The positive control substances used for the mutagenicity tests were ethyl methanesulphonate (EMS) and 2-acetylaminofluorene (AAF), both of which were obtained from Koch-Light Laboratories, Colnbrook, Buckinghamshire.

Thymidine and trifluorothymidine (TFT) came from Sigma Chemical Company Limited, Poole, Dorset. Hypoxanthine was obtained from PL Biochemicals, Milwaukee, Wisconsin, U.S.A., while methotrexate came from the Lederle Laboratories Division of Cyanamid of Great Britain, Gosport, Hampshire, U.K.

The polychlorinated biphenyl mixture Aroclor 1254 was received from Analabs Incorporated, Newhaven, Connecticut, U.S.A.

The vehicle control dimethylsulphoxide (DMSO) was obtained from BDH Chemicals Limited, Poole, England.

Cells

L5178Y mouse lymphoma cells heterozygous at the TK locus were obtained from D. Clive, Research Triangle Park, North Carolina, U.S.A.

Cell Growth and Maintenance

Fischer's medium (10x) and horse serum were obtained from Gibco Laboratories Limited, Paisley, while donor horse serum was obtained from Flow Laboratories Limited, Irvine, Scotland. The basic cell growth medium (F_0P) consisted of Fischer's medium supplemented with penicillin (100 units/ml), streptomycin (100 µg/ml), sodium bicarbonate (1.125 g/l), sodium pyruvate (2 mM), pluronic acid (0.5% w/v) and glutamine (2 mM).

The addition of horse serum (10% v/v) to F_0P gave $F_{10}P$ which was the medium used for all cell growth and culture maintenance.

The addition of F_0P of donor horse serum (20% v/v) and sodium pyruvate to 4 mM gave cloning medium (CM), the medium required for colony formation during experiments.

During routine culture maintenance, cells were grown in Nunclon Delta tissue culture flasks at 37°C and in an atmosphere of 5% CO_2:95% air (v/v). Cell density was calculated daily using a Neubauer haemocytometer and cultures diluted with $F_{10}P$ to a concentration of 3×10^5 cells/ml.

The Activation Mixture

Animals

Male SD rats weighing 250-300 g were injected once i.p. with Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml) at a dosage of 500 mg/kg 5 days before they were killed. The animals were allowed drinking water continuously, but food was withheld 16 h before they were killed.

Preparation of the 9000 g Supernatant Fluid from Livers

Freshly killed animals were thoroughly swabbed with 70% alcohol, the abdomen opened and liver removed, taking special care not to cut into the gastrointestinal tract. The livers were collected in tared beakers containing ice-cold homogenisation medium. The medium used was 0.15 M-KCl.

The beakers were weighed and the collected livers transferred to the homogenisation vessel. A volume of ice-cold 0.15 M-KCl, equivalent to 3 times the weight of the liver was added to the vessel and the livers chopped using long handled scissors. The chopped livers were homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) was rotating at about 1200 rpm. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun to give 9000 g for 10 min at $0^{\circ}C$-$+2^{\circ}C$. The supernatant fluid was decanted leaving behind a thick pellet of (mainly) whole cells, nuclei and mitchondria. Post-mitochondrial supernatant fluids were freshly prepared in sufficient quantity for this experiment.

Preparation of the "S-9 Mix"

Ice-cold 0.05 M-phosphate buffer, pH 7.4, was added to preweighed NADP and glucose-6-phosphate, etc., as follows, to give a final concentation in the "S-9 mix" of:

NADP-di-Na salt	4 mM	(= 3.366 mg/ml)
Glucose-6-phosphate-di-Na salt	5 mM	(= 1.521 mg/ml)
$MgCl_2.6H_2O$	8 mM	(= 1.626 mg/ml)
KCl	33 mM	(= 2.460 mg/ml)

This solution was immediately filter sterilised by passage through 0.45 µm Millipore filter and mixed with the liver 9000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

Toxicity Test

An initial toxicity test was carried out in the absence of S-9 mix in order to select doses of the chemical for mutation test.

10 ml samples of mouse lymphoma culture, containing 3×10^6 cells were exposed to one of 5 doses of compound.

Dilutions were carried out as follows:

		Final Concentration
(1)	Stock solution HE 1003 at 106 mg/ml in DMSO	1060 µg/ml
(2)	0.1 ml (1) +0.9 ml DMSO	106 µg/ml
(3)	0.1 ml (2) +0.9 ml DMSO	10.6 µg/ml
(4)	0.1 ml (3) +0.9 ml DMSO	1 µg/ml
(5)	0.1 ml (4) +0.9 ml DMSO	0.1 µg/ml

Incubation was carried out for 3 h at 37°C. After incubation, the cells were harvested by centrifugation at 1000 r.p.m. in the MSE Chilspin centrifuge for 5 min, then

resuspended in 10 ml $F_{10}P$. Each harvested culture was transferred to a 75 cm^2 Nunclon Delta tissue culture flask, gassed thoroughly with 5% CO_2:95% air (v/v) and left to grow at 37°C.

Cell density was measured by counting with a Neubauer haemocytometer each day for the next 3 days until the toxic effects of the chemical could be estimated.

Mutation Test

3×10^6 exponentially growing $TK^{+/-}$ mouse lymphoma L5178Y cells were dispensed to sterile, plastic universal bottles in 5 ml $F_{10}P$. 5 ml F_0P was added to give 10 ml of cells in F_5P medium.

The concentrations of test substance HE 1003 used in the mutation test were finally fixed at 250 µg/ml, 83 µg/ml, 25 µg/ml, 8.3 µg/ml and 2.5 µg/ml.

0.1 ml of appropriate test compound solution was added to each bottle, followed by 1 ml of freshly prepared S-9 mix if required. All bottles were incubated on a gyrotary shaker at 37°C, 150 rpm, for 3 h.

After the incubation period, the cells were harvested by centrifugation at 1000 rpm for 5 min, then resuspended in 10 ml $F_{10}P$. A sample was taken from each of these cell suspensions and plated in soft agar to determine cell survival.

For mutant colony selection in trifluorothymidine (TFT) cloning medium, 2 x 5 ml samples of suspension were dispensed into plastic universal bottles and the cells harvested by centrifugation. Each pellet was resuspended in 19.5 ml cloning medium without agar. Molten agar was added to give a concentration of 0.25% and the medium dispensed into 3 x 58 mm Petri dishes as for the survival assay. Hence, 6 TFT plates were prepared from each test culture.

After gelling and equilibration in a 95% air:5% CO_2 (v/v) atmosphere, the plates were sealed in boxes and incubated at 37°C. Colonies were counted manually 7-10 days later.

Calculations of Experimental Results

The number of cells plated per dish for the survival assay and for estimation of the number of TFT resistant mutants can be calculated from the figures given above.

For the survival assay, routinely 0.1 ml of 3 x 10^5 cells/ml + 4.9 ml $F_{10}P$ gave a 1:50 dilution.

0.1 ml of diluted culture of 6 x 10^3 cells/ml were added to 21 ml CM + agar and the suspension divided between 3 petri dishes.

=) 200 cells were plated per dish.

For estimation of the number of TFT resistant mutants, 1.5 x 10^6 cells in total (5 ml of 3 x 10^5 cells/ml) were added to 21 ml CM and the suspension divided between 3 petri dishes.

=) 5 x 10^5 cells were plated per dish.

Survival Assay

A survival assay was carried out immediately after exposure of cells with test compound. Cells were diluted as follows:

(A) 0.1 ml cell suspension + 4.9 ml $F_{10}P$ medium
0.1 ml (A) + 21 ml cloning medium containing 0.25% agar.

7 ml samples were poured into each of 3 x 58 mm tissue culture petri dishes and left to set at 4°C. After gelling, the plates were equilibrated with 5% CO_2:95% air (v/v) in plastic containers which were then sealed and incubated at 37°C for 10 days.

Expression of Genetic Damage

For 3 days after exposure to mutagen, cells were allowed to multiply in $F_{10}P$ medium. Cultures were counted each day using a Neubauer haemocytometer and diluted to 3×10^5 cells/ml.

On the third day after exposure, the cells were adjusted to 3×10^5 cells/ml and a sample taken for the survival assay for which it was diluted as follows:

(A) 0.1 ml cell suspension + 4.9 ml $F_{10}P$.
0.1 ml (A) + 21 ml cloning medium containing 0.25% agar.

The cloning medium suspension was then dispensed to 3 x 58 mm petri dishes and the plates processed as for the day 0 survival estimation.

If X is the average no. of survivors/plate.
Y is the average no. of TFT mutants/plate.

=) No. of survivors per ml of suspension after 3 days incubation

$$= (X) \times \frac{3 \times 10^5}{2 \times 10^2} = (X) \times 1,500$$

=) No. of TFT mutants per ml of suspension after 3 days incubation

$$= (Y) \times \frac{3 \times 10^5}{5 \times 10^5} = (Y) \times 0.6$$

=) No. of TFT mutants/10^5 survivors

$$= \frac{0.6Y}{1500X} \times 10^5$$

If the numbers of cells plated in any particular experiment differed from those above, then the actual experiment figure has been quoted under the appropriate Table of Results.

RESULTS AND DISCUSSION

In an initial toxicity test carried out in the absence of S-9, the mouse lymphoma culture treated with 1060 µg/ml HE 1003 was completely killed (Table 1). Between 106 µg/ml and 0.1 µg/ml HE 1003, cultures survived and the top dose chosen for the first mutation test was 100 µg/ml test compound.

The criterion used by Clive (Clive et al., 1979) to describe a positive result in this test was a doubling of mutation frequency over the solvent treated (spontaneous) value. In addition to this, a dose response shown by the induced mutation frequencies of at least 2 concentrations of test compound was considered necessary before a significant positive result could be recorded.

The mutation frequencies obtained in these tests are all compared with the frequency induced by the solvent control. In this laboratory, several dozens of mouse lymphoma mutation tests have been carried out during 1980. On only 2 occasions was the mutation frequency of the culture treated with DMSO greater than 10 mutants per 10^5 survivors. Mutation frequencies of between 4 to 9 mutants per 10^5 survivors were found in almost all other control cultures. The mutation frequency of 18 obtained for one of the DMSO-treated cultures in the first experiment in the absence of S-9 mix (Table 2) was, therefore, extremely unusual. This value was discounted when the results of the experiment were being evaluated.

Three mutation tests were carried out in the absence of S-9 (Tables 2 to 4). In the first experiment (Table 2), both the highest and lowest doses of the test compound induced mutation frequencies greater than twice that of one of the two DMSO-treated cultures and absolute increases in TFT^+ colonies were recorded at these concentrations. No dose response was

apparent in this experiment. Also, the other DMSO-treated culture showed a mutation frequency which was unacceptably high. In these circumstances, the results of this experiment were considered to be of dubious value.

Two further mutation tests were carried out in the absence of S-9 mix. The first of these experiments was carried out over the dose range of 15.6 to 250 µg/ml HE 1003. In this test a negative result was obtained, as none of the mutation frequencies induced by a dose of HE 1003 fulfilled the first criterion for a positive response required by Clive (Table 3). The third experiment retained the top dose of 250 µg/ml but HE 1003 was tested at concentrations as low as 2.5 µg/ml. At 8.3 µg/ml, a mutation frequency 3 times that of the solvent control culture was obtained, accompanied by an absolute increase in the number of TFT^+ colonies recorded (Table 4). At no other dose was this the case. In conclusion, therefore, although increases in the number of TFT^+ mutant colonies compared to the solvent control were seen at isolated doses in 2 out of 3 experiments (or one out of 2 valid experiments) in the absence of S-9, the evidence was not sufficient for the compound to be termed potentially mutagenic in these conditions.

In 3 mutation experiments carried out in the presence of S-9 mix (Tables 5 to 7) there was no evidence for any mutagenic behaviour by HE 1003. At 10 µg/ml in the first test (Table 5), HE 1003 induced a mutation frequency exactly twice that of the solvent control culture. No dose effect was observed, however, in this experiment. A high mutation frequency was recorded at 62.5 µg/ml test compound in the second test (Table 6). There was no increase in the number of mutants and it is clear that the high frequency was an artefact of a poor day 3 survival culture which was infected.

CONCLUSION

In the absence of S-9 mix, increased numbers of TFT^+ mutants compared to solvent control were observed at isolated dose levels in 2 out of 3 experiments, but this evidence was not sufficient to lead to the suspicion that HE 1003 is a mutagen. Similarly, in the presence of S-9 mix, no evidence of mutagenic activity was obtained for this compound. It was concluded, therefore, that compound HE 1003 showed no mutagenic activity in the mouse lymphoma forward mutation assay.

REFERENCES

(1) Clive, D., Flamm, W.G., Machesko, M.R., and Bernheim N.J., (1972). A mutational assay system using the thymidine kinase locus in mouse lymphoma cells. Mutation Research, 16, 77-87.

(2) Clive, D. and Spector, J.F.S., (1977). Laboratory Procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Handbook of Mutagenicity Test Procedures, Kilbey et al., Eds. Elsevier Scientific Publishing Co., 161-173.

(3) Clive, D., Johnstone, K.O., Spector, J.F.S., Batson, A.G. and Brown, M.M.M. (1979). Validation and characterization of the L5178Y $TK^{+/-}$ mouse lymphoma mutagen assay system. Mutation Research, 59, 61-108.

TABLE 1

Cytotoxicity Test in the Absence of S-9

Project No.:	703837	Substance:	HE 1003
Contractor:	Bayer Study No. HE 1003/015	Activation:	None
Operator:	Carole Ross	Liver preparation date:	None
Date of test:	19 August 1980	Cell culture batch:	TK 2.8.5

Substance Quantity µg/ml	Haemocytometer Counts Total Visible Cells x 10^6		
	Day 1	Day 2	Day 3
HE 1003			
1060*	-	-	-
106*	6.3	23.2	61.6
10.6	7.8	24.5	62.4
1	7.0	29.8	80.0
0.1	8.6	35.7	94.8
DMSO	6.8	35.8	97.5
DMSO	9.5	34.7	85.8
DMSO	8.5	30.9	99.0

Selected dose range for mutation test:

100, 33, 10, 3.3, 1 µg/ml

*pptn of compound on contact with incubation medium.

pptn = precipitation

TABLE 2

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 26 August 1980

Substance: HE 1003
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.9.1

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % Control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003								
100*	82,81,63	75	74	171,inf, inf	171	52,24,57, 40,47,29	42	10
33	99,91,117	102	100	150,136, 201	162	21,38,38, inf,10,10	25	6
10	101,94,70	88	86	128,134,185	149	17,16,24, inf,inf,inf	19	5
3.3	126,109, 121	119	117	164,182,208	185	20,28,29, 24,20,17	23	5
1	85,59,77	74	73	186,190,160	179	47,80,64, 71,69,66	66	15
EMS 400	107,95,85	96	94	161,195,184	180	147,178,206 158,224,169	180	40
200	96,86,107	96	94	217,232,252	234	103,138,122, 109,118,110	117	20
DMSO	60,46,51	52	51	180,163,164	169	90,95,65, 76,70,66	77	18
DMSO	90,103,113	102	100	163,227,222	204	40,24,25 21,19,18	25	5

* pptn of compound on contact with incubation medium
inf = infected

TABLE 3

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 30 September 1980

Substance: HE 1003
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.9.2

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % Control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003								
250*	134,159 165	153	87	123,124,142	130	5,15,inf, 7,7,10	9	3
125*	112,96,83	97	55	148,inf,inf,	148	10,inf,inf, inf,14,15	13	4
62.5	132,142, 142	139	79	170,186,123	160	12,8,17 13,17,19	14	4
31.2	92,119,117	109	62	173,129,175	159	14,25,inf, 21,18,14	18	5
15.6	54,84,77	72	41	155,inf,134	145	19,17,34 29,21,24	24	7
EMS								
400	111,115,98	108	61	89,107,134	110	102,44,90, 89,89,89	84	31
400	98,149,126	124	70	97,115,128	113	74,102,147, 90,120 inf	108	38
200	119,109,72	100	57	189,inf,16	103	116,91,69, 50,98,69	82	32
DMSO	186,184, 158	176	100	86,105,94	95	15,8,11, 8,11,19	12	5

* potn of compound on contact with incubation medium
inf = infected

TABLE 4

Mutation Test - In the Absence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 14 October 1980

Substance: HE 1003
Activation: None
Liver preparation date: None
Cell culture batch: TK 2.11.2

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % Control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003								
250*	55,40,31	42	43	125,129,116	123	inf	inf	-
83	55,73,66	65	67	213,206,209	209	35,38,27, 41,33,43	36	7
25	99,103,71	91	94	170,171,177	173	44,38,30, 43,31,38	37	9
8.3	22,38,17	26	27	179,175,164	173	95,69,85, 81,79,79	81	18
2.5	48,75,63	62	64	119,131,174	141	38,28,29, 34,43,40	35	10
EMS								
400	61,46,59	55	57	103,119,138	120	126,106,144, 140,129,116	126	42
200	6,16,18	13	13	103,116,101	107	36,52,31, 81,96,97	66	24
DMSO	86,100,106	97	100	171,203,162	179	17,27,22, 23,29,30	25	6

* pptn of compound on contact with incubation medium
inf = infected

TABLE 5

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 26 August 1980

Substance: HE 1003
Activation: Aroclor-induced Fischer rat
Liver preparation date: 13.8.80
Cell culture batch: TK 2.9.1

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % Control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003								
100*	114,134, 116	121	105	183,155,inf	169	19,34,22, 15,33,13	23	5
33	113,91,101	102	89	109,123,121	118	10,11,11,10, 10,12	12	4
10	58,55,55	56	49	127,158,162	149	23,16,48, inf,inf,inf	29	8
3.3	145,122, 110	126	110	148,inf,132	140	18,25,19, 26,17,21	21	6
1	135,152, 147	145	126	142,107,168	139	15,8,8, 10,13,12	11	3
AAF								
100	28,31,19	26	22	104,97,89	97	69,30,48, 64,18,30	43	18
50	38,46,28	37	32	128,112,153	131	87,52,76 89,61,7	73	22
DMSO	98,86,91	92	80	212,inf,inf	212	1,24,37, 24,14,19	20	4
DMSO	98,138,110	115	100	220,284,144	216	15,19,20, 19,22,13	18	3

* pptn of compound on contact with incubation medium
inf = infected

TABLE 6

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 30 September 1980

Substance: HE 1003
Activation: Aroclor-induced Fischer rat
Liver preparation date: 13.8.80
Cell culture batch: TK 2.9.2

Substance Quantity µg/ml	Day 3				TFT^+ Colonies/ 10^5 Survivors
	Survival		TFT^+		
	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003					
250*	182,178,148	169	35,23,28, 25,31,26	28	7
125*	183,186,158	176	12,20,13, 9,11,21	14	3
62.5	5,inf,inf	5	12,14,12, 8,5,inf	10	80
31.2	240,215,184	213	15,20,24, 18,23,29	22	4
15.6	137,144,209	163	15,19,inf 22,26,inf	21	5
AAF					
100	inf,0,1	-	0,0,0	-	-
100	5,2,1	-	0,0,0	-	-
50	102,102,76	93	16,46,44 25,32,44	35	15
DMSO	191,258,inf	225	23,25,25 23,18,22	23	4

inf = infected
Day 0 plates did not grow
*pptn of compound on contact with incubation medium

TABLE 7

Mutation Test - In the Presence of S-9
Plate Counts

Project No.: 703837
Contractor: Bayer Study No. HE 1003/015
Operator: Carole Ross
Date of test: 14 October 1980

Substance: HE 1003
Activation: Aroclor-induced Fischer rat
Liver preparation date: 13.8.80
Cell culture batch: TK 2.11.2

Substance Quantity µg/ml	Day 0			Day 3				TFT+ Colonies/ 10^5 Survivors
				Survival		TFT+		
	Colonies/ dish	Average	Survival % Control	Colonies/ dish	Average	Colonies/ dish	Average	
HE 1003 250*	68,67,44	60	88	36,32,58	42	10,3,2, 2,4,4	4	4
83	75,76,68	73	107	113,105,113	110	6,9,5, 9,8,12	8	3
25	68,71,57	65	96	202,190,206	199	34,37,36, 21,28,31	31	6
8.3	68,82,81	77	113	190,167,158	172	30,38,21, 30,29,18	28	6
2.5	74,60,59	64	94	166,167,168	167	13,11,15, 10,16,17	14	3
AAF 100	3,3,3,	3	4	51,54,70	58	52,64,inf 24,32,15	37	26
50	6,6,2	5	7	118,94,109	107	25,49,49, 45,61,29	43	16
DMSO	62,78,64	68	100	179,184,147	170	22,11,21, 10,22,15	16	4

* pptn of compound on contact with incubation medium
inf = infected

Bericht Nr. R 2098

Exemplar-Nr.:

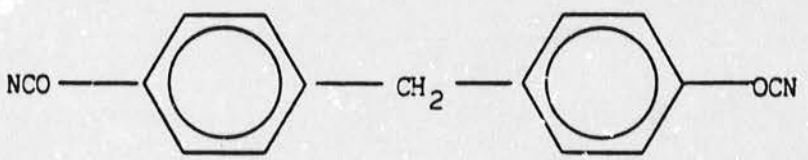
Anhang zu:

Testing the mutagenic activity of compound HE 1003 in the mouse lymphoma mutation assay.

IRI Project No. 703837

Autoren: D.B.McGregor, W.J.Harris und C.A.Ross

Bei HE 1003 handelt es sich um Desmodur 44 V 20 (MDI), ein technisches Gemisch mit der Summenformel $C_{15}H_{10}O_2N_2$ und der Struktur



Die Studie trug die BAYER Studien Nr.:

DESMODUR 44 V 20 / 015

Wuppertal, den 5. Oktober 1981

(Dr. B. Herbold)
Monitor

CERTIFICATE OF AUTHENTICITY

THIS IS TO CERTIFY that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 3 (Month) 24 (Day) 92 (Year) Marcia Tubolino Camera Operator

Place Syracuse (City) New York (State)

AMTEK corp